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(54) Tumor necrosis factor related receptor, TR6

(57) TR6 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing TR6 polypeptides and polynucleotides in the design of protocols for the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease., among others and diagnostic assays for such conditions.

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Description

This application is a continuation-in-part application of U.S. Serial No: 08/853,684, filed May 9, 1997, which claims the benefit of U.S. Provisional Application No: 60/041,230, filed March 14, 1997.

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to Tumor Necrosis Factor Related family, hereinafter referred to as TR6. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

Many biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intracellular response.

For example, tumor necrosis factors (TNF) alpha and beta are cytokines which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-ligands, the "TNF-receptor" superfamily. So far, nine members of the TNF ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized.

Among the ligands there are included TNF-α, lymphotoxin-α (LT-α, also known as TNF-β), LT-β (found in complex heterotrimer LT-α2-β), FasL, CD40L, CD27L, CD30L, 4-1BBL, OX40L and nerve growth factor (NGF)). The superfamily of TNF receptors includes the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-1BB, OX40, low affinity p75 and NGF-receptor (Meager, A., Biologicals, 22:291-295 (1994)).

Many members of the TNF-ligand superfamily are expressed by activated T-cells, implying that they are necessary for T-cell interactions with other cell types which underlie cell ontogeny and functions. (Meager, A., supra).

Considerable insight into the essential functions of several members of the TNF receptor family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., et al., Nature 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglubulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R.C. et al., Science 259:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innovation of peripheral structures (Lee, K.F. et al, Cell 69:737 (1992)).

TNF and LT-α are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT-α, acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT-α are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmuine disease, AIDS and graft-host rejection (Beutler, B. and Von Huffel, C., Science 264:667-668 (1994)). Mutations in the p55 Receptor cause increased susceptibility to microbial infection.

Moreover, an about 80 amino acid domain near the C-terminus of TNFR1 (P55) and Fas was reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia et al., Cell 74:845 (1993)).

The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence biological activity, both normally and in disease states. In particular, there is a need to isolate and characterize novel members of the TNF receptor family.

This indicates that these receptors have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further receptors which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to TR6 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such TR6 polypeptides and polynucleotides. Such uses include the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with TR6 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate TR6 activity or levels.

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"TR6" refers, among others, to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2, or an allelic variant thereof.

"Receptor Activity" or "Biological Activity of the Receptor" refers to the metabolic or physiologic function of said TR6 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said TR6.

"TR6 gene" refers to a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation,

iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al.*, Nucleic Acids Research (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al.*, *J Molec Biol* (1990) 215:403).

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5 or 3 terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Polypeptides of the Inventi n

In one aspect, the present invention relates to TR6 polypeptides. The TR6 polypeptides include the polypeptides of SEQ ID NOS:2 and 4; as well as polypeptides comprising the amin acid sequence of SEQ ID NO:2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within TR6 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO: 2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Preferably TR6 polypeptides exhibit at least one biological activity of the receptor.

The TR6 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the TR6 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned TR6 polypeptides. As with TR6 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of TR6 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of TR6 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate receptor activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the receptor, including antigenic activity. Among the most preferred fragment is that having the amino acid sequence of SEQ ID NO: 4. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The TR6 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to TR6 polynucleotides. TR6 polynucleotides include isolated polynucleotides which encode the TR6 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, TR6 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO: 1 encoding a TR6 polypeptide of SEQ ID NO: 2, and polynucleotides having the particular sequences of SEQ ID NOS: 1 and 3. TR6 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the TR6 polypeptide of SEQ ID NO:2 over its entire length, and a polynucleotide that is at least 80% identical to that having SEQ ID NO:1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under TR6 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplifica-

tion or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such TR6 polynucleotides.

TR6 of the invention is structurally related to other proteins of the Tumor Necrosis Factor Related family, as shown by the results of sequencing th cDNA encoding human TR6. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide numbers 94 to 1329) encoding a polypeptide of 411 amino acids of SEQ ID NO:2. The amino acid sequence of Table 1 (SEQ ID NO:2) has about 58% identity (using GAP (From GCG)) in 411 amino acid residues with DR4, the receptor for the ligand TRAIL. (Pan,G., O'Rourke,K., Chinnaiyan,A.M., Gentz,R., Ebner,R., Ni,J. and Dixit,V.M., Science 276, 111-113 (1997)). The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 70% identity (using GAP (from GCG)) in 1335 nucleotide residues with DR4, the receptor for the ligand TRAIL. TR6 contains a death domain (amino acids 290 to 324 in SEQ ID NO:2) which is 64% identical to the death domain of the human Death receptor 4 (DR4) (Pan,G., O'Rourke,K., Chinnaiyan,A.M., Gentz,R., Ebner,R., Ni,J. and Dixit,V.M., Science 276, 111-113 (1997)), 35.7% identical to the death domain of the human Death receptor 3 (DR3) (A.M. Chinnaiyan, et al, Science 274 (5289), 990-992 (1996)), 32.7% identical to the death domain of human TNFR-1, and 19.6% identical to the death domain of CD95 (Fas) (I. Cascino, J. Immunol. 154 (6), 2706-2713 (1995)).

Table 1[a]

```
   1  CTTTGCGCCC ACAAAATACA CCGACGATGC CCGATCTACT TTAAGGGCTG

  51  AAACCCACGG GCCTGAGAGA CTATAAGAGC GTTCCCTACC GCCATGGAAC

 101  AACGGGGACA GAACGCCCCG GCCGCTTCGG GGGCCCGGAA AAGGCACGGC

 151  CCAGGACCCA GGGAGGCGCG GGGAGCCAGG CCTGGGCCCC GGGTCCCCAA

 201  GACCCTTGTG CTCGTTGTCG CCGCGGTCCT GCTGTTGGTC TCAGCTGAGT

 251  CTGCTCTGAT CACCCAACAA GACCTAGCTC CCCAGCAGAG AGCGGCCCCA

 301  CAACAAAAGA GGTCCAGCCC CTCAGAGGGA TTGTGTCCAc cTGGACACCA

 351  TATCTCAGAA GACGGTAGAG ATTGCATCTC CTGCAAATAT gGACAGGACT

 401  ATAGCACTCA aTGGAATGAC CTCCTTTTCT GCTTGCGCTG CACCAGGTGT

 451  GATTCAGGTG AAGTGGAGCT AAGTCCCTGC ACCACGACCA GAAACACAGT

 501  GTGTCAGTGC GAAGAAgGCA CCTTCCGGGA AGAAGATTCT CCTGAGATGT
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551  GCCGGAAGTG CCGCACAGGG TGTCCCAgAG GGATGGTCAA GGTCGGTGAT

601  TGTACACCCT GGAGTGACAT CGAATGTGTC CACAAAGAAT CAGGCATCAT

651  CATAgGAGTC ACAGTTGCAG CCGTAGTCTT GATTGTGGCT GTGTTTGTTT

701  GCaAgTCTTT ACTGTGGAAg AAAGTCCTTC CTTACCTGAA AGGCATCTGC

751  TCAGGTGGTG GTGGGGACCC TGAGCGTGTG GACAGAAGcT CACAACGACc

801  TGGGGCTGAG GACAATGTCC TCAATGAGAT CGTGAGTATC TTGCAGCCCA

851  CCCAGGTCCC TGAGCAGGAA ATGGAAGTCC AGGAGCCAGC AGAGCCAACA

901  GGTGTCAACA TGTTGTCCCC CGGGGAGTCA GAGCATCTGC TGGAACCGGC

951  AGAAGCTGAA AGGTCTCAGA GGAGGAGGCT GCTGGTTCCA GCAAATGAAG

1001 GTGATCCCAC TGAGACTCTG AGACAGTGCT TCGATGACTT TGCAGACTTG

1051 GTGCCCTTTG ACTCCTGGGA gCCgCTCATG AGGAAGTTGG GCCTCATGGA

1101 CAATgAGATa aaGGTGGCTA AAGCTGAGGC AGCGGGCCAC AGGGACACCT

1151 TGTACACGAT GCTGATAAAG TGGGTCAACA AAACCGGGCG AGATGCCTCT

1201 GTCCACACCC TGCTGGATGC CTTGGAGACG CTGGGAGAGA GACTTGCCAA

1251 GCAGAAGATT GAGGACCACT TGTTGAGCTC TGGAAAGTTC ATGTATCTAG

1301 AAGGTAATGC AGACTCTGCC ATGTCCTAAG TGTGATTCTC TTCAGGAAGT

1351 CAGACCTTCC CTGGTTTACC TTTTTTCTGG AAAAAGCCCA ACTGGACTCC

1401 AGTCAGTAGG AAAGTGCCAC AATTGTCACA TGACCGGTAC TGGAAGAAAC

1451 TCTCCCATCC AACATCACCC AGTGGATGGA ACATCCTGTA ACTTTTCACT

1501 GCACTTGGCA TTATTTTTAT AAGCTGAATG TGATAATAAG GACACTATGG
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1551  AAATGTCTGG ATCATTCCGT TTGTGCGTAC TTTGAgATTT GGTTTGGGAT
1601  GTCATTGTTT TCACAGCACT TTTTTATCCT AATGTAAATG CTTTATTTAT
1651  TTATTTGGGC TACATTGTAA gATCCATCTA CACAGTCGTT GTCCGACTTC
1701  ACTTGATACT ATATGATATG AACCTTTTTT GGGTGGGGGG TGCGGGGCAg
1751  TTCACTCTGT CTCCCAGGCT GGAGTGCAAT GGTGCAATCT TGGCTCACTA
1801  TAGCCTTGAC CTCTCAGGCT CAAGCGATTC TCCCACCTCA GCCATCCAAA
1851  TAGCTGGGAC CACAGGTGTG CACCACCACG CCCGGCTAAT TTTTTGTATT
1901  TTGTCTAgAT ATAGGGGCTC TCTATGTTGC TCAGGGTGGT CTCgAATTCC
1951  TGGAcTCAAG CAGTCTGCCC ACcTCAGAcT CCCAAAGCGG TGGAATTAGA
2001  GGCGTGAGCC CCCATGcTTG gCCTTACcTT TcTACTTTTA TAATTCTGTA
2051  TGTTATTATT TTATGAACAT GAAGAAACTT TAGTAAATGT ACTTGTTTAC
2101  ATAGTTATGT GAATAGATTA GATAAACATA AAAGGAGGAG ACATACAATG
2151  GGGGAAGAAG AAGAAGTCCC CTGTAAGATG TCACTGTcTG GGTTCCAGCC
2201  CTCCCTCAGA TGTACTTTGG CTTCAATGAT TGGCAACTTC TACAGGGGCC
2251  AGTCTTTTGA ACTGGACAAC CTTACAAGTA TATGAGTATT ATTTATAGGT
2301  AGTTGTTTAC ATATGAGTCG GGACCAAAGA GAACTGGATC CACGTGAAGT
2351  CCTGTGTGTG GCTGGTCCCT ACCTGGGCAG TcTCATTTGC ACCCATAGCC
2401  CCCATCTATG GACAGGCTGG GACAGAGGCA GATGGGTTAG ATCACACATA
2451  ACAATAGGGT CTATGTCATA TCCCAAGTGA ACTTGAGCCC TGTTTGGGCT
2501  CAGGAGATAG AAGACAAAAT CTGTCTCCCC ACGTCTGCCA TGGCATCAAG
2551  GGGGAAGAGT AGATGGTGCT tGAGAATGGT GTGAAATGGT TGCCATCTCA
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2601  GGAGTAGATG GCCCGGCTCA CTTCTGGTTA TCtGTCACCC TGAGCCCAtG

2651  AGCTGCcTTT TAGGGTACAG ATTGCCTACT TGAGGACCTT GGCCGCTCTG

2701  TAAGCATCTG ACTCATCTCA GAAATGTCAA TTCTTAAACA CTGTGGCAAC

2751  AGGACCTAGA ATGGCTGACG CATTAAGGTT TTCTTcTTGT GTCCTGTTCT

2801  ATTAtTGTTT TAAGACCTCA GTAACCATTT CAGCCTCTTT CCAGCAAACC

2851  CTTCTCCATA GTATTTCAGT CATGGAAGGA TCATTTATGC AGGTAGTCAT

2901  TCCAGGAGTT TTTGGTCTTT TCTGTCTCAA GGCATTGTGT GTTTTGTTCC

2951  GGGACTGGTT TGGGTGGGAC AAAGTTAGAA TTGCCTGAAG ATcAcACATT

3001  CAGACTGTTtG TGTCTGTGGA GTTTTAGGAG TGGGGGGTGA CCTTTcTGGT

3051  CTTtGcAcTT CCATCcTcTC CCAcTTCCAT cTGGCATCCC CACGcGTTGT

3101  CCCcTGCAcT TcTGGAAGGC ACAGGGTGCT GCTGCTTCCT GGTCTTTGCC

3151  TTTGCTGGGC cTTCTGTGCA GGACGCTCAG CCTCAGGGCT CAGAAGGTGC

3201  CAGTCCGGTC CCAGGTCCCT TGTCCCTTCC ACAGAGGCCT TCcTAGAAGA

3251  TGCATCTAGA GTGTCAGCCT TATCAGTGTT TAAGATTTTT CTTTTATTTT

3301  TAATTTTTTT GAGACAGAAT CTCACTCTCT CGCCCAGGCT GGAGTGCAAC

3351  GGTACGATCT TGGCTCAGTG CAACCTCCGC CTCCTGGGTT CAAGCGATTC

3401  TCGTGCCTCA GCCTCCGGAG TAGCTGGGAT TGCAGGCACC CGCCACCACG

3451  CCTGGCTAAT TTTTGTATTT TTAGTAGAGA CGGGGTTTCA CCATGTTGGT

3501  CAGGCTGGTC TCGAACTCCT GACCTCAGGT GATCCACNTT GGCCTCCGAA

3551  AGTGCTGGGa tatacaaggc GTGAGCCACC AGCCAGGCCA AGATATTNTT
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3601  NTAAAGNNAG CTTCOGGANG ACATGAAATA ANGGGGGGTT TTGTTGTTTA

3651  GTAACATTNG GCTTTGATAT ATCCCCAGGC CAAATNGCAN GNGACACAGG

3701  ACAGCCATAG TATAGTGTGT CACTCGTGGT TGGTGTCCTT TCATGGTTCT

3751  GCCCTGTCAA AGGTCCCTAT TTGAAATGTG TTATAATACA AACAAGGAAG

3801  CACATTGTGT ACAAAATACT TATGTATTTA TGAATCCATG ACCAAATTAA

3851  ATATGAAACC TTATATAAAA AAAAAAAAAA A
```

[a] A nucleotide sequence of a human TR6. (SEQ ID NO: 1).

Table 2[b]

```
  1  Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg Lys   16

 17  Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro Gly Pro   32

 33  Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu Leu Leu   48

 49  Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln   64

 65  Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu Gly Leu   80

 81  Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser   96

 97  Cys Lys Tyr Gly Gln Asp Tyr Ser Thr Gln Trp Asn Asp Leu Leu Phe  112

113  Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro  128

129  Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe  144

145  Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys  160

161  Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile  176

177  Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly Val Thr Val Ala  192

193  Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp  208
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209	Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly	224
225	Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp	240
241	Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro	256
257	Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn	272
273	Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala	288
289	Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu Gly Asp	304
305	Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val	320
321	Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp	336
337	Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr	352
353	Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala	368
369	Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu	384
385	Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met	400
401	Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser End	411

[b] An amino acid sequence of a human TR6. (SEQ ID NO: 2).

One polynucleotide of the present invention encoding TR6 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human of human thymus stromal cells, monocytes, peripheral blood lymphocytes, primary dendritic, and bone marrow cells using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature*, (1992) *355*:632-634; Adams, M.D., *et al., Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding TR6 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 94 to 1329 of SEQ ID NO:1), or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of TR6 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, Proc *Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding TR6 variants comprising the amino acid sequence

of TR6 polypeptide of Table 1 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination. Among the preferred polynucleotides of the present invention is contained in Table 3 (SEQ ID NO: 3) encoding the amino acid sequence of Table 4 (SEQ ID NO: 4).

Table 3[c]

```
   1  ATGACCTCCT TTTCTGCTTG CGCTGCACCA GGTGTGATTC AGGTGAAGTG

  51  GAGCTAAGTC CCTGCACCAC GACCAGAAAC ACAGTGTGTC AGTGCGAAGA

 101  AgGCACCTTC CGGGAAGAAG ATTCTCCTGA GATGTGCCGG AAGTGCCGCA

 151  CAGGGTGTCC CAgAGGGATG GTCAAGGTCG GTGATTGTAC ACCCTGGAGT

 201  GACATCGAAT GTGTCCACAA AGAATCAGGC ATCATCATAg GAGTCACAGT
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 251  TGCAGCCGTA GTCTTGATTG TGGCTGTGTT TGTTTGCaAg TCTTTACTGT

 301  GGAAgAAAGT CCTTCCTTAC CTGAAAGGCA TCTGCTCAGG TGGTGGTGGG

 351  GACCCTGAGC GTGTGGACAG AAGcTCACAA CGACcTGGGG CTGAGGACAA

 401  TGTCCTCAAT GAGATCGTGA GTATCTTGCA GCCCACCCAG GTCCCTGAGC

 451  AGGAAATGGA AGTCCAGGAG CCAGCAGAGC CAACAGGTGT CAACATGTTG

 501  TCCCCCGGGG AGTCAGAGCA TCTGCTGGAA CCGGCAGAAG CTGAAAGGTC

 551  TCAGAGGAGG AGGCTGCTGG TTCCAGCAAA TGAAGGTGAT CCCACTGAGA

 601  CTCTGAGACA GTGCTTCGAT GACTTTGCAG ACTTGGTGCC CTTTGACTCC

 651  TGGGAgCCgC TCATGAGGAA GTTGGGCCTC ATGGACAATg AGATaaaGGT

 701  GGCTAAAGCT GAGGCAGCGG GCCACAGGGA CACCTTGTAC ACGATGCTGA

 751  TAAAGTGGGT CAACAAAACC GGGCGAGATG CCTCTGTCCA CACCCTGCTG

 801  GATGCCTTGG AGACGCTGGG AGAGAGACTT GCCAAGCAGA AGATTGAGGA

 851  CCACTTGTTG AGCTCTGGAA AGTTCATGTA TCTAGAAGGT AATGCAGACT

 901  CTGCCATGTC CTAAGTGTGA TTCTCTTCAG GAAGTCAGAC CTTCCCTGGT

 951  TTACCTTTTT TCTGGAAAAA GCCCAACTGG ACTCCAGTCA GTAGGAAAGT

1001  GCCACAATTG TCACATGACC GGTACTGGAA GAAACTCTCC CATCCAACAT

1051  CACCCAGTGG AT
```

[c] A partial nucleotide sequence of a human TR6. (SEQ ID NO: 3).

Table 4[d]

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   1  DLLFCLRCTR CDSGEVELSP CTTTRNTVCQ CEEGTFREED SPEMCRKCRT
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 51  GCPRGMVKVG DCTPWSDIEC VHKESGIIIG VTVAAVVLIV AVFVCKSLLW

101  KKVLPYLKGI CSGGGGDPER VDRSSQRPGA EDNVLNEIVS ILQPTQVPEQ

151  EMEVQEPAEP TGVNMLSPGE SEHLLEPAEA ERSQRRRLLV PANEGDPTET

201  LRQCFDDFAD LVPFDSWEPL MRKLGLMDNE IKVAKAEAAG HRDTLYTMLI

251  KWVNKTGRDA SVHTLLDALE TLGERLAKQK IEDHLLSSGK FMYLEGNADS

301  AMS*
```

[a] A partial amino acid sequence of a human TR6. (SEQ ID NO: 4).

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof, including that of SEQ ID NO:3, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding TR6 and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the TR6 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

In one embodiment, to obtain a polynucleotide encoding TR6 polypeptide comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof, including that of SEQ ID
NO: 3, and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Thus in another aspect, TR6 polynucleotides of the present invention further include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having SEQ ID NO: 1 or a fragment thereof, including that of SEQ ID NO:3. Also included with TR6 polypeptides are polypeptide comprising amino acid sequence encoded by nucleotide sequence obtained by the above hybridization condition. Stringent hybridization conditions are as defined above or alternatively conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran

mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEX 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the TR6 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If TR6 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

TR6 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of TR6 polynucleotides for use as diagnostic reagents. Detection of a mutated form of TR6 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of TR6. Individuals carrying mutations in the TR6 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled TR6 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising TR6 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, through detection of mutation in the TR6 gene by the methods described.

In additi n, chr nic and acute inflammation, arthritis, septicemia, autoimmune diseases (.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease

syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of TR6 polypeptide or TR6 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an TR6, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

The 3' untranslated region of TR6 matches the 295 bp nucleotide sequence of a mapped EST (Genbank ID: D20151). This EST has been mapped by the Whitehead Institute to chromosome 8, 97.68 cR from the top of the Chromosome 8 linkage group

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the TR6 polypeptides. The term "immunospecific" means that the antibodies have substantiall greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the TR6 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against TR6 polypeptides may also be employed to treat chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with TR6 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschierosis, and Alzheimers disease, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering TR6 polypeptide via a vector directing expression of TR6 polynucleotide *in vivo* in order to induce such an immunological response to pro-

duce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a TR6 polypeptide wherein the composition comprises a TR6 polypeptide or TR6 gene. The vaccine formulation may further comprise a suitable carrier. Since TR6 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulati ns suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation instonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

We have now discovered that TL2 of SEQ ID NO: 5 (otherwise known as TRAIL, Immunity (6):673-682 (1995)) is a ligand of TR6. Thus, the TR6 polypeptide of the present invention, and one of its ligands, TL2 may be employed in a screening process for compounds which bind the receptor, or its ligand, and which activate (agonists) or inhibit activation of (antagonists) the receptor polypeptide of the present invention, or its ligand TL2. Thus, polypeptides of the invention may be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

TR6 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate TR6 on the one hand and which can inhibit the function of TR6 or remove TR6 expressing cells on the other hand. Antagonists, or agents which remove TR6 expressing cells, may be employed for a variety of therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease. Agonists can be employed for therapeutic and prophylactic purposes for such conditions responsive to activation of T cells and other components of the immune system, such as for treatment of cancer and AIDS. However, agonists can also be employed for inappropriate stimulation of T cells and other components of the immune system which leads to down modulation of immune activity with therapeutic or prophylactic application for conditions such , as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, , Bone diseases,, atheroschlerosis, and Alzheimers disease.

Candidate compounds may be identified using assays to detect compounds which inhibit binding of TL2 to TR6 in either cell-free or cell based assays. Suitable cell-free assays may be readily determined by one of skill in the art. For example, an ELISA format may be used in which purified TR6, or a purified derivative of TR6, containing the extracellular domain of TR6, is immobilized on a suitable surface, either directly or indirectly (e.g., via an antibody to TR6) and candidate compounds are identified by their ability to block binding of purified TL2 to TR6. The binding of TL2 to TR6 could be detected by using a label directly or indirectly associated with TL2. Suitable detection systems include the streptavidin horseradish peroxidase conjugate, or direct conjugation by a tag, e.g., fluorescein. Conversely, purified TL2 may be immobilized on a suitable surface, and candidate compounds identified by their ability to block binding of purified TR6 to TL2. The binding of TR6 to TL2 could be detected by using a label directly or indirectly associated with TR6. Many other assay formats are possible that use the TR6 protein and its ligands.

Suitable cell based assays may be readily determined by one of skill in the art. In general, such screening procedures involve producing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells expressing the receptor (or cell membrane containing the expressed receptor) are then contacted with a known ligand, such as TL2, or test compound to observe binding, or stimulation or inhibition of a functional response. The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the receptor is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor, such as the ligand TL2. Further, these assays may test whether the candidat compound results in a signal generated by activation of the receptor or its ligand (e.g. TL2)using detection systems appropriate to the cells bearing the receptor or its ligand

and fusion proteins thereof at their surfaces. Typical fusion partners include fusing the extracellular domain f the receptor or ligand with the intracellular tyrosine kinase domain of a second receptor. Inhibitors of activation are generally assayed in the presence of a known agonist, such as the ligand TL2, and the effect on activation by the agonist by the presence of the candidate compound is observed. Standard methods for conducting such screening assays are well understood in the art.

Examples of potential TR6 antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligand of the TR6, e.g., a fragment of the ligand TL2, or small molecules which bind to the receptor, or its ligand, but do not elicit a response, so that the activity of the receptor is prevented. Examples of potential TR6 agonists include antibodies that bind to TR6, its ligand, such as TL2, or derivatives thereof, and small molecules that bind to TR6. These agonists will elicit a response mimicking all or part of the response induced by contacting the native ligand.

The nucleotide sequence of TL2 (SEQ ID NO:5) (published by Immunex Research and Development Corporation, Seattle, Washington as TNF-related apoptosis-inducing ligand (TRAIL) TWiley SR, et al. Immunity (6):673-682 (1995)) is as follows.

```
   1  CCTCACTGAC TATAAAAGAA TAGAGAAGGA AGGGCTTCAG TGACCGGCTG

  51  CCTGGCTGAC TTACAGCAGT CAGACTCTGA CAGGATCATG GCTATGATGG

 101  AGGTCCAGGG GGGACCCAGC CTGGGACAGA CCTGCGTGCT GATCGTGATC

 151  TTCACAGTGC TCCTGCAGTC TCTCTGTGTG GCTGTAACTT ACGTGTACTT

 201  TACCAACGAG CTGAAGCAGA TGCAGGACAA GTACTCCAAA AGTGGCATTG
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 251  CTTGTTTCTT AAAAGAAGAT GACAGTTATT GGGACCCCAA TGACGAAGAG

 301  AGTATGAACA GCCCCTGCTG GCAAGTCAAG TGGCAACTCC GTCAGCTCGT

 351  TAGAAAGATG ATTTTGAGAA CCTCTGAGGA AACCATTTCT ACAGTTCAAG

 401  AAAAGCAACA AAATATTTCT CCCCTAGTGA GAGAAAGAGG TCCTCAGAGA

 451  GTAGCAGCTC ACATAACTGG GACCAGAGGA AGAAGCAACA CATTGTCTTC

 501  TCCAAACTCC AAGAATGAAA AGGCTCTGGG CCGCAAAATA AACTCCTGGG

 551  AATCATCAAG GAGTGGGCAT TCATTCCTGA GCAACTTGCA CTTGAGGAAT

 601  GGTGAACTGG TCATCCATGA AAAAGGGTTT TACTACATCT ATTCCCAAAC

 651  ATACTTTCGA TTTCAGGAGG AAATAAAAGA AAACACAAAG AACGACAAAC

 701  AAATGGTCCA ATATATTTAC AAATACACAA GTTATCCTGA CCCTATATTG

 751  TTGATGAAAA GTGCTAGAAA TAGTTGTTGG TCTAAAGATG CAGAATATGG

 801  ACTCTATTCC ATCTATCAAG GGGGAATATT TGAGCTTAAG GAAAATGACA

 851  GAATTTTTGT TTCTGTAACA AATGAGCACT TGATAGACAT GGACCATGAA

 901  GCCAGTTTTT TCGGGGCCTT TTTAGTTGGC TAACTGACCT GGAAAGAAAA

 951  AGCAATAACC TCAAAGTGAC TATTCAGTTT TCAGGATGAT ACACTATGAA

1001  GATGTTTCAA AAAATCTGAC CAAAACAAAC AAACAGAAAA CAGAAAACAA

1051  AAAAACCTCT ATGCAATCTG AGTAGAGCAG CCACAACCAA AAAATTCTAC

1101  AACACACACT GTTCTGAAAG TGACTCACTT ATCCCAAGAA AATGAAATTG

1151  CTGAAAGATC TTTCAGGACT CTACCTCATA TCAGTTTGCT AGCAGAAATC

1201  TAGAAGACTG TCAGCTTCCA AACATTAATG CAATGGTTAA CATCTTCTGT
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1251  CTTTATAATC TACTCCTTGT AAAGACTGTA GAAGAAAGCG CAACAATCCA

1301  TCTCTCAAGT AGTGTATCAC AGTAGTAGCC TCCAGGTTTC CTTAAGGGAC

1351  AACATCCTTA AGTCAAAAGA GAGAAGAGGC ACCACTAAAA GATCGCAGTT

1401  TGCCTGGTGC AGTGGCTCAC ACCTGTAATC CCAACATTTT GGGAACCCAA

1451  GGTGGGTAGA TCACGAGATC AAGAGATCAA GACCATAGTG ACCAACATAG

1501  TGAAACCCCA TCTCTACTGA AAGTGCAAAA ATTAGCTGGG TGTGTTGGCA

1551  CATGCCTGTA GTCCCAGCTA CTTGAGAGGC TGAGGCAGGA GAATCGTTTG

1601  AACCCGGGAG GCAGAGGTTG CAGTGTGGTG AGATCATGCC ACTACACTCC

1651  AGCCTGGCGA CAGAGCGAGA CTTGGTTTCA AAAAAAAAAA AAAAAAAAAA

1701  CTTCAGTAAG TACGTGTTAT TTTTTTCAAT AAAATTCTAT TACAGTATGT

1751  CAAAAAAAAA AAAAAAAAA
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The amino acid sequence of TL2 (SEQ ID NO:6) (published by Immunex Research and Development Corporation, Seattle, Washington as TNF-related apoptosis-inducing ligand (TRAIL) TWiley SR, et al. Immunity (6):673-682 (1995)) is as follows:

```
 1  Met Ala Met Met Glu Val Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys  16

17  Val Leu Ile Val Ile Phe Thr Val Leu Leu Gln Ser Leu Cys Val Ala  32

33  Val Thr Tyr Val Tyr Phe Thr Asn Glu Leu Lys Gln Met Gln Asp Lys  48

49  Tyr Ser Lys Ser Gly Ile Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr  64

65  Trp Asp Pro Asn Asp Glu Glu Ser Met Asn Ser Pro Cys Trp Gln Val  80

81  Lys Trp Gln Leu Arg Gln Leu Val Arg Lys Met Ile Leu Arg Thr Ser  96
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97	Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser Pro	112
113	Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly	128
129	Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu	144
145	Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly	160
161	His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile	176
177	His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe	192
193	Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln	208
209	Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys	224
225	Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr	240
241	Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile	256
257	Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu Ala	272
273	Ser Phe Phe Gly Ala Phe Leu Val Gly End	281

Prophylactic and Therapeutic Methods

This invention provides methods of treating abnormal conditions such as, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, related to both an excess of and insufficient amounts of TR6 activity.

If the activity of TR6 is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the TR6, or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of TR6 polypeptides still capable of binding the ligand in competition with endogenous TR6 may be administered. Typical embodiments of such competitors comprise fragments of the TR6 polypeptide.

In still another approach, expression of the gene encoding endogenous TR6 can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al*, Nucleic Acids Res (1979) 6:3073; Cooney *et al.*, Science (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of TR6 and its activity, several approaches are also

available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates TR6, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of TR6 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of TR6 polypeptides in combination with a suitable pharmaceutical carrier.

Formulation and Administration

Peptides, such as the soluble form of TR6 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Examples

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

Example 1

Two ESTs (EST#1760054 and EST#1635744) with sequence similarity to the human TNF receptor were discovered in a commercial EST database. Analysis of the two nucleotide sequences (3,466 bp and 2,641 bp respectively), revealed each was a partial sequence of the complete cDNA sequence, overlapping, with 100% identity, 2,226 bp at the nucleotide level. Together, the two sequences encompassed the complete predicted cDNA sequence of 3,881 bp, and encoded an open reading frame for a novel member of the TNF receptor superfamily and named TR6. The predicted protein is 411 amino acids long with a hydrophobic membrane spanning region indicating that at least one form of TR6 is expressed as a membrane bound protein. Comparison of TR6 protein sequence, with other TNF receptor family proteins indicates that it has two of the cysteine-rich repeats characteristic of the extracellular domains of this family, and an intracellular death domain,

Northern blot of TR6.

Various tissues and cell lines were screened for mRNA expression by Northern blot. RNA was prepared from cells and cell lines using Tri-Reagent (Molecular Research Center Inc., Cincinnati, OH), run in denaturing agarose gels (Sambrook et al., Molecular Cloning: a laboratory manual, 2nd Ed. Cold Spring Harbor Lab Press, NY (1989)) and transfered to Zeta-probe nylon membrane (Biorad, Hercules, CA.) via vacuum blotting in 25mM NaOh for 90 min. After neutralization for 5-10 minutes with 1M tris-HCl, pH 7.5 containing 3M NaCl, the blots were prehybridized with 50% formamide, 8% dextran sulfate, 6XSSPE, 0.1%SDS and 100mg/ml of sheared and dentured salmon sperm DNA for at least 30 min. At 42°C. cDNA probes were labeled with 32P-CTP by random priming (Statagene, La Jolla, CA), briefly denatured with 0.25M NaOH and added to the prehybridization solution. After a further incubation for at least 24h at 42°C, the blots were washed in high stringency conditions and exposed to X-ray film.

Very high expression of TR6 RNA was detected in aortic endothelial cells. High expression was also detected in monocytes. Low expression was detected in bone marrow and CD4+ activated PBLs. Very low, but detectable levels of TR6 RNA was expressed in CD19+ PBLs, CD8+ PBLs (both activated and unstimulated), and unstimulated CD4+ PBLs.

In hematopoietic cell lines, low levels of TR6 RNA was expressed in HL60 (promyelocyte), KG1a (promyeloblast) and KG1 (myeloblast) cell lines. Very low but detectable levels of TR6 RNA was expressed in U937 (monoblast) and THP-1 (monocyte) cell lines.

The major RNA form is 3.8 kb in size.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: SmithKline Beecham Corporation

(ii) TITLE OF THE INVENTION: TUMOR NECROSIS FACTOR RELATED RECEPTOR, TR6

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: SmithKline Beecham, Corporate Intellectual Property
(B) STREET: Two New Horizons Court
(C) CITY: Brentford
(D) COUNTY: Middlesex
(E) COUNTRY: United Kingdom
(F) POST CODE: TW8 9EP

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: 22-AUGUST-1997
(C) CLASSIFICATION: Unknown

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/853,684
(B) FILING DATE: 09-MAY-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: THOMPSON, Clive Beresford
(B) GENERAL AUTHORISATION NUMBER: 5630
(C) REFERENCE/DOCKET NUMBER: GH-50008-1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: +44 181 975 6347
(B) TELEFAX: +44 181 975 6294
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3,881 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CTTTGCGCCC ACAAAATACA CCGACGATGC CCGATCTACT TTAAGGGCTG AAACCCACGG 60
GCCTGAGAGA CTATAAGAGC GTTCCCTACC GCCATGGAAC AACGGGGACA GAACGCCCCG 120
GCCGCTTCGG GGGCCCGGAA AAGGCACGGC CCAGGACCCA GGGAGGCGCG GGGAGCCAGG 180
CCTGGGCCCC GGGTCCCCAA GACCCTTGTG CTCGTTGTCG CCGCGGTCCT GCTGTTGGTC 240
TCAGCTGAGT CTGCTCTGAT CACCCAACAA GACCTAGCTC CCCAGCAGAG AGCGGCCCCA 300
CAACAAAAGA GGTCCAGCCC CTCAGAGGGA TTGTGTCCAC CTGGACACCA TATCTCAGAA 360
GACGGTAGAG ATTGCATCTC CTGCAAATAT GGACAGGACT ATAGCACTCA ATGGAATGAC 420
CTCCTTTTCT GCTTGCGCTG CACCAGGTGT GATTCAGGTG AAGTGGAGCT AAGTCCCTGC 480
ACCACGACCA GAAACACAGT GTGTCAGTGC GAAGAAGGCA CCTTCCGGGA AGAAGATTCT 540
CCTGAGATGT GCCGGAAGTG CCGCACAGGG TGTCCCAGAG GGATGGTCAA GGTCGGTGAT 600
TGTACACCCT GGAGTGACAT CGAATGTGTC CACAAAGAAT CAGGCATCAT CATAGGAGTC 660
ACAGTTGCAG CCGTAGTCTT GATTGTGGCT GTGTTTGTTT GCAAGTCTTT ACTGTGGAAG 720
AAAGTCCTTC CTTACCTGAA AGGCATCTGC TCAGGTGGTG GTGGGGACCC TGAGCGTGTG 780

GACAGAAGCT CACAACGACC TGGGGCTGAG GACAATGTCC TCAATGAGAT CGTGAGTATC 840
TTGCAGCCCA CCCAGGTCCC TGAGCAGGAA ATGGAAGTCC AGGAGCCAGC AGAGCCAACA 900
GGTGTCAACA TGTTGTCCCC CGGGGAGTCA GAGCATCTGC TGGAACCGGC AGAAGCTGAA 960
AGGTCTCAGA GGAGGAGGCT GCTGGTTCCA GCAAATGAAG GTGATCCCAC TGAGACTCTG 1020
AGACAGTGCT TCGATGACTT TGCAGACTTG GTGCCCTTTG ACTCCTGGGA GCCGCTCATG 1080
AGGAAGTTGG GCCTCATGGA CAATGAGATA AAGGTGGCTA AAGCTGAGGC AGCGGGCCAC 1140
AGGGACACCT TGTACACGAT GCTGATAAAG TGGGTCAACA AAACGGGCCG AGATGCCTCT 1200
GTCCACACCC TGCTGGATGC CTTGGAGACG CTGGGAGAGA GACTTGCCAA GCAGAAGATT 1260
GAGGACCACT TGTTGAGCTC TGGAAAGTTC ATGTATCTAG AAGGTAATGC AGACTCTGCC 1320
ATGTCCTAAG TGTGATTCTC TTCAGGAAGT CAGACCTTCC CTGGTTTACC TTTTTTCTGG 1380
AAAAAGCCCA ACTGGACTCC AGTCAGTAGG AAAGTGCCAC AATTGTCACA TGACCGGTAC 1440
TGGAAGAAAC TCTCCCATCC AACATCACCC AGTGGATGGA ACATCCTGTA ACTTTTCACT 1500
GCACTTGGCA TTATTTTTAT AAGCTGAATG TGATAATAAG GACACTATGG AAATGTCTGG 1560
ATCATTCCGT TTGTGCGTAC TTTGAGATTT GGTTTGGGAT GTCATTGTTT TCACAGCACT 1620
TTTTTATCCT AATGTAAATG CTTTATTTAT TTATTTGGGC TACATTGTAA GATCCATCTA 1680
CACAGTCGTT GTCCGACTTC ACTTGATACT ATATGATATG AACCTTTTTT GGGTGGGGGG 1740
TGCGGGGCAG TTCACTCTGT CTCCCAGGCT GGAGTGCAAT GGTGCAATCT TGGCTCACTA 1800
TAGCCTTGAC CTCTCAGGCT CAAGCGATTC TCCCACCTCA GCCATCCAAA TAGCTGGGAC 1860
CACAGGTGTG CACCACCACG CCCGGCTAAT TTTTTGTATT TTGTCTAGAT ATAGGGGCTC 1920
TCTATGTTGC TCAGGGTGGT CTCGAATTCC TGGACTCAAG CAGTCTGCCC ACCTCAGACT 1980
CCCAAAGCGG TGGAATTAGA GGCGTGAGCC CCCATGCTTG GCCTTACCTT TCTACTTTTA 2040
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TATGAGTATT ATTTATAGGT AGTTGTTTAC ATATGAGTCG GGACCAAAGA GAACTGGATC 2340
CACGTGAAGT CCTGTGTGTG GCTGGTCCCT ACCTGGGCAG TCTCATTTGC ACCCATAGCC 2400
CCCATCTATG GACAGGCTGG GACAGAGGCA GATGGGTTAG ATCACACATA ACAATAGGGT 2460
CTATGTCATA TCCCAAGTGA ACTTGAGCCC TGTTTGGGCT CAGGAGATAG AAGACAAAAT 2520
CTGTCTCCCC ACGTCTGCCA TGGCATCAAG GGGGAAGAGT AGATGGTGCT TGAGAATGGT 2580
GTGAAATGGT TGCCATCTCA GGAGTAGATG GCCCGGCTCA CTTCTGGTTA TCTGTCACCC 2640
TGAGCCCATG AGCTGCCTTT TAGGGTACAG ATTGCCTACT TGAGGACCTT GGCCGCTCTG 2700
TAAGCATCTG ACTCATCTCA GAAATGTCAA TTCTTAAACA CTGTGGCAAC AGGACCTAGA 2760
ATGGCTGACG CATTAAGGTT TTCTTCTTGT GTCCTGTTCT ATTATTGTTT TAAGACCTCA 2820
GTAACCATTT CAGCCTCTTT CCAGCAAACC CTTCTCCATA GTATTTCAGT CATGGAAGGA 2880
TCATTTATGC AGGTAGTCAT TCCAGGAGTT TTTGGTCTTT TCTGTCTCAA GGCATTGTGT 2940

GTTTTGTTCC GGGACTGGTT TGGGTGGGAC AAAGTTAGAA TTGCCTGAAG ATCACACATT 3000
CAGACTGTTG TGTCTGTGGA GTTTTAGGAG TGGGGGGTGA CCTTTCTGGT CTTTGCACTT 3060
CCATCCTCTC CCACTTCCAT CTGGCATCCC CACGCGTTGT CCCCTGCACT TCTGGAAGGC 3120
ACAGGGTGCT GCTGCTTCCT GGTCTTTGCC TTTGCTGGGC CTTCTGTGCA GGACGCTCAG 3180
CCTCAGGGCT CAGAAGGTGC CAGTCCGGTC CCAGGTCCCT TGTCCCTTCC ACAGAGGCCT 3240
TCCTAGAAGA TGCATCTAGA GTGTCAGCCT TATCAGTGTT TAAGATTTTT CTTTTATTTT 3300
TAATTTTTTT GAGACAGAAT CTCACTCTCT CGCCCAGGCT GGAGTGCAAC GGTACGATCT 3360
TGGCTCAGTG CAACCTCCGC CTCCTGGGTT CAAGCGATTC TCGTGCCTCA GCCTCCGGAG 3420
TAGCTGGGAT TGCAGGCACC CGCCACCACG CCTGGCTAAT TTTTGTATTT TTAGTAGAGA 3480
CGGGGTTTCA CCATGTTGGT CAGGCTGGTC TCGAACTCCT GACCTCAGGT GATCCACNTT 3540
GGCCTCCGAA AGTGCTGGGA TATACAAGGC GTGAGCCACC AGCCAGGCCA AGATATTNTT 3600
NTAAAGNNAG CTTCCGGANG ACATGAAATA ANGGGGGGTT TTGTTGTTTA GTAACATTNG 3660
GCTTTGATAT ATCCCCAGGC CAAATNGCAN GNGACACAGG ACAGCCATAG TATAGTGTGT 3720
CACTCGTGGT TGGTGTCCTT TCATGGTTCT GCCCTGTCAA AGGTCCCTAT TTGAAATGTG 3780
TTATAATACA AACAAGGAAG CACATTGTGT ACAAAATACT TATGTATTTA TGAATCCATG 3840
ACCAAATTAA ATATGAAACC TTATATAAAA AAAAAAAAAA A 3881

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 411 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg Lys
1 5 10 15
Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro Gly Pro
20 25 30
Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu Leu Leu
35 40 45
Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln

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     50                  55                  60
Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu Gly Leu
 65                  70                  75                  80
Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser
                 85                  90                  95
Cys Lys Tyr Gly Gln Asp Tyr Ser Thr Gln Trp Asn Asp Leu Leu Phe
            100                 105                 110
Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro
        115                 120                 125
Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe
    130                 135                 140
Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys
145                 150                 155                 160
Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile
                165                 170                 175
Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly Val Thr Val Ala
            180                 185                 190
Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp
        195                 200                 205
Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly
    210                 215                 220
Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp
225                 230                 235                 240
Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro
                245                 250                 255
Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn
            260                 265                 270
Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala
        275                 280                 285
Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu Gly Asp
    290                 295                 300
Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val
305                 310                 315                 320
Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp
                325                 330                 335
Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr
            340                 345                 350
Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala
        355                 360                 365
Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu
    370                 375                 380
Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met
```

385 390 395 400

Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser End

405 410 411

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1062 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATGACCTCCT TTTCTGCTTG CGCTGCACCA GGTGTGATTC AGGTGAAGTG GAGCTAAGTC 60
CCTGCACCAC GACCAGAAAC ACAGTGTGTC AGTGCGAAGA AGGCACCTTC CGGGAAGAAG 120
ATTCTCCTGA GATGTGCCGG AAGTGCCGCA CAGGGTGTCC CAGAGGGATG GTCAAGGTCG 180
GTGATTGTAC ACCCTGGAGT GACATCGAAT GTGTCCACAA AGAATCAGGC ATCATCATAG 240
GAGTCACAGT TGCAGCCGTA GTCTTGATTG TGGCTGTGTT TGTTTGCAAG TCTTTACTGT 300
GGAAGAAAGT CCTTCCTTAC CTGAAAGGCA TCTGCTCAGG TGGTGGTGGG GACCCTGAGC 360
GTGTGGACAG AAGCTCACAA CGACCTGGGG CTGAGGACAA TGTCCTCAAT GAGATCGTGA 420
GTATCTTGCA GCCCACCCAG GTCCCTGAGC AGGAAATGGA AGTCCAGGAG CCAGCAGAGC 480
CAACAGGTGT CAACATGTTG TCCCCCGGGG AGTCAGAGCA TCTGCTGGAA CGGCAGAAG 540
CTGAAAGGTC TCAGAGGAGG AGGCTGCTGG TTCCAGCAAA TGAAGGTGAT CCCACTGAGA 600
CTCTGAGACA GTGCTTCGAT GACTTTGCAG ACTTGGTGCC CTTTGACTCC TGGGAGCCGC 660
TCATGAGGAA GTTGGGCCTC ATGGACAATG AGATAAAGGT GGCTAAAGCT GAGGCAGCGG 720
GCCACAGGGA CACCTTGTAC ACGATGCTGA TAAAGTGGGT CAACAAAACC GGGCGAGATG 780
CCTCTGTCCA CACCCTGCTG GATGCCTTGG AGACGCTGGG AGAGAGACTT GCCAAGCAGA 840
AGATTGAGGA CCACTTGTTG AGCTCTGGAA AGTTCATGTA TCTAGAAGGT AATGCAGACT 900
CTGCCATGTC CTAAGTGTGA TTCTCTTCAG GAAGTCAGAC CTTCCCTGGT TTACCTTTTT 960
TCTGGAAAAA GCCCAACTGG ACTCCAGTCA GTAGGAAAGT GCCACAATTG TCACATGACC 1020
GGTACTGGAA GAAACTCTCC CATCCAACAT CACCCAGTGG AT 1062

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 303 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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Asp Leu Leu Phe Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val
 1               5                  10                  15
Glu Leu Ser Pro Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu
            20                  25                  30
Glu Gly Thr Phe Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys
        35                  40                  45
Arg Thr Gly Cys Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro
    50              55                  60
Trp Ser Asp Ile Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly
65                  70                  75                  80
Val Thr Val Ala Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys
                85                  90                  95
Ser Leu Leu Trp Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser
            100                 105                 110
Gly Gly Gly Gly Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro
        115                 120                 125
Gly Ala Glu Asp Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro
    130                 135                 140
Thr Gln Val Pro Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro
145                 150                 155                 160
Thr Gly Val Asn Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu
                165                 170                 175
Pro Ala Glu Ala Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala
            180                 185                 190
Asn Glu Gly Asp Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe
        195                 200                 205
Ala Asp Leu Val Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu
    210                 215                 220
Gly Leu Met Asp Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly
225                 230                 235                 240
His Arg Asp Thr Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr
                245                 250                 255
Gly Arg Asp Ala Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu
            260                 265                 270
Gly Glu Arg Leu Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser
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275 280 285

Gly Lys Phe Met Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser

290 295 300

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1769 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCTCACTGAC TATAAAAGAA TAGAGAAGGA AGGGCTTCAG TGACCGGCTG CCTGGCTGAC 60
TTACAGCAGT CAGACTCTGA CAGGATCATG GCTATGATGG AGGTCCAGGG GGGACCCAGC 120
CTGGGACAGA CCTGCGTGCT GATCGTGATC TTCACAGTGC TCCTGCAGTC TCTCTGTGTG 180
GCTGTAACTT ACGTGTACTT TACCAACGAG CTGAAGCAGA TGCAGGACAA GTACTCCAAA 240
AGTGGCATTG CTTGTTTCTT AAAAGAAGAT GACAGTTATT GGGACCCCAA TGACGAAGAG 300
AGTATGAACA GCCCCTGCTG GCAAGTCAAG TGGCAACTCC GTCAGCTCGT TAGAAAGATG 360
ATTTTGAGAA CCTCTGAGGA AACCATTTCT ACAGTTCAAG AAAAGCAACA AAATATTTCT 420
CCCCTAGTGA GAGAAAGAGG TCCTCAGAGA GTAGCAGCTC ACATAACTGG GACCAGAGGA 480
AGAAGCAACA CATTGTCTTC TCCAAACTCC AAGAATGAAA AGGCTCTGGG CCGCAAAATA 540
AACTCCTGGG AATCATCAAG GAGTGGGCAT TCATTCCTGA GCAACTTGCA CTTGAGGAAT 600
GGTGAACTGG TCATCCATGA AAAAGGGTTT TACTACATCT ATTCCCAAAC ATACTTTCGA 660
TTTCAGGAGG AAATAAAAGA AAACACAAAG AACGACAAAC AAATGGTCCA ATATATTTAC 720
AAATACACAA GTTATCCTGA CCCTATATTG TTGATGAAAA GTGCTAGAAA TAGTTGTTGG 780
TCTAAAGATG CAGAATATGG ACTCTATTCC ATCTATCAAG GGGGAATATT TGAGCTTAAG 840
GAAAATGACA GAATTTTTGT TTCTGTAACA AATGAGCACT TGATAGACAT GGACCATGAA 900
GCCAGTTTTT TCGGGGCCTT TTTAGTTGGC TAACTGACCT GGAAAGAAAA AGCAATAACC 960
TCAAAGTGAC TATTCAGTTT TCAGGATGAT ACACTATGAA GATGTTTCAA AAAATCTGAC 1020
CAAAACAAAC AAACAGAAAA CAGAAAACAA AAAAACCTCT ATGCAATCTG AGTAGAGCAG 1080
CCACAACCAA AAAATTCTAC AACACACACT GTTCTGAAAG TGACTCACTT ATCCCAAGAA 1140
AATGAAATTG CTGAAAGATC TTTCAGGACT CTACCTCATA TCAGTTTGCT AGCAGAAATC 1200
TAGAAGACTG TCAGCTTCCA AACATTAATG CAATGGTTAA CATCTTCTGT CTTTATAATC 1260
TACTCCTTGT AAAGACTGTA GAAGAAAGCG CAACAATCCA TCTCTCAAGT AGTGTATCAC 1320
AGTAGTAGCC TCCAGGTTTC CTTAAGGGAC AACATCCTTA AGTCAAAAGA GAGAAGAGGC 1380
ACCACTAAAA GATCGCAGTT TGCCTGGTGC AGTGGCTCAC ACCTGTAATC CCAACATTTT 1440
GGGAACCCAA GGTGGGTAGA TCACGAGATC AAGAGATCAA GACCATAGTG ACCAACATAG 1500
TGAAACCCCA TCTCTACTGA AAGTGCAAAA ATTAGCTGGG TGTGTTGGCA CATGCCTGTA 1560
GTCCCAGCTA CTTGAGAGGC TGAGGCAGGA GAATCGTTTG AACCCGGGAG GCAGAGGTTG 1620

CAGTGTGGTG AGATCATGCC ACTACACTCC AGCCTGGCGA CAGAGCGAGA CTTGGTTTCA 1680
AAAAAAAAAA AAAAAAAAAA CTTCAGTAAG TACGTGTTAT TTTTTTCAAT AAAATTCTAT 1740
TACAGTATGT CAAAAAAAAA AAAAAAAAA 1769

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 281 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Met Met Glu Val Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys
1 5 10 15
Val Leu Ile Val Ile Phe Thr Val Leu Leu Gln Ser Leu Cys Val Ala
20 25 30
Val Thr Tyr Val Tyr Phe Thr Asn Glu Leu Lys Gln Met Gln Asp Lys
35 40 45
Tyr Ser Lys Ser Gly Ile Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr
50 55 60
Trp Asp Pro Asn Asp Glu Glu Ser Met Asn Ser Pro Cys Trp Gln Val
65 70 75 80
Lys Trp Gln Leu Arg Gln Leu Val Arg Lys Met Ile Leu Arg Thr Ser
85 90 95
Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser Pro
100 105 110
Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly
115 120 125
Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu
130 135 140
Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly
145 150 155 160
His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile
165 170 175
His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe
180 185 190
Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln
195 200 205
Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys

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    210                 215                 220
Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr
225                 230                 235                 240
Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile
                245                 250                 255
Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu Ala
            260                 265                 270
Ser Phe Phe Gly Ala Phe Leu Val Gly
        275                 280
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Claims

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the TR6 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence.

2. The polynucleotide of claim 1 which is DNA or RNA.

3. The polynucleotide of claim 1 wherein said nucleotide sequence is at least 80% identical to that contained in SEQ ID NO:1.

4. The polynucleotide of claim 3 wherein said nucleotide sequence comprises the TR6 polypeptide encoding sequence contained in SEQ ID NO:1.

5. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.

6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a TR6 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.

7. A host cell comprising the expression system of claim 6.

8. A process for producing a TR6 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.

9. A process for producing a cell which produces a TR6 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a TR6 polypeptide.

10. A TR6 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.

11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.

12. An antibody immunospecific for the TR6 polypeptide of claim 10.

13. A method for the treatment of a subject in need of enhanced activity or expression of TR6 polypeptide of claim 10 comprising:

    (a) administering to the subject a therapeutically effective amount of an agonist to said receptor; and/or
    (b) providing to the subject an isolated polynucleotide comprising a nucleotide sequenc that has at least 80%

identity to a nucleotide sequence encoding the TR6 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said polypeptide activity *in vivo*.

14. A method for the treatment of a subject having need to inhibit activity or expression of TR6 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an antagonist to said receptor; and/or
(b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said receptor; and/or
(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said receptor for its ligand.

15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of TR6 polypeptide of claim 10 in a subject comprising:

(a) determining the presence or absence of a mutation in the nucleotide sequence encoding said TR6 polypeptide in the genome of said subject; and/or
(b) analyzing for the presence or amount of the TR6 polypeptide expression in a sample derived from said subject.

16. A method for identifying agonists to TR6 polypeptide of claim 10 comprising:

(a) contacting a cell which produces a TR6 polypeptide with a candidate compound; and
(b) determining whether the candidate compound effects a signal generated by activation of the TR6 polypeptide.

17. An agonist identified by the method of claim 16.

18. The method for identifying antagonists to TR6 polypeptide of claim 10 comprising:

(a) contacting said a cell which produces a TR6 polypeptide with an agonist; and
(b) determining whether the signal generated by said agonist is diminished in the presence of a candidate compound.

19. An antagonist identified by the method of claim 18.

20. A recombinant host cell produced by the process of claim 9 or a membrane thereof expressing a TR6 polypeptide.

(19) Europäisches Patentamt
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(54) Tumor necrosis factor related receptor, TR6

(57) TR6 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing TR6 polypeptides and polynucleotides in the design of protocols for the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease., among others and diagnostic assays for such conditions.

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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 97 31 0562

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
P,X	SHERIDAN J P ET AL: "Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors" SCIENCE, vol. 277, 8 August 1997, pages 818-821, XP002075799 * the whole document * ---	1-12, 15-20	C12N15/12 C07K14/715 C07K16/28 A61K38/17 C12Q1/68 G01N33/68
P,X	PAN G ET AL: "AN ANTAGONIST DECOY RECEPTOR AND A DEATH DOMAIN-CONTAINING RECEPTOR FOR TRAIL" SCIENCE, vol. 277, 8 August 1997, pages 815-818, XP002065147 * the whole document * ---	1-12,15, 16,20	
A	WO 97 01633 A (IMMUNEX CORP) 16 January 1997 * figures 1,2; example 13 * ---		
A	EP 0 510 691 A (OSAKA BIOSCIENCE INST) 28 October 1992 see tha claims * abstract; figures 1,2,7; examples 1,2 * --- -/--		

TECHNICAL FIELDS SEARCHED (Int.Cl.6)
C07K
A61K
C12N
C12Q
G01N

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

Although claims 13 and 14 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Place of search	Date of completion of the search	Examiner
THE HAGUE	28 August 1998	Oderwald, H

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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Application Number
EP 97 31 0562

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	CHINNAIYAN A M ET AL: "SIGNAL TRANSDUCTION BY DR3, A DEATH DOMAIN-CONTAINING RECEPTOR RELATED TO TNFR-1 AND CD95" SCIENCE, vol. 274, no. 5289, 8 November 1996, pages 990-992, XP000676685 * the whole document *		TECHNICAL FIELDS SEARCHED (Int.Cl.6)

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